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Synthesis of the starfish ganglioside LLG-3 tetrasaccharide

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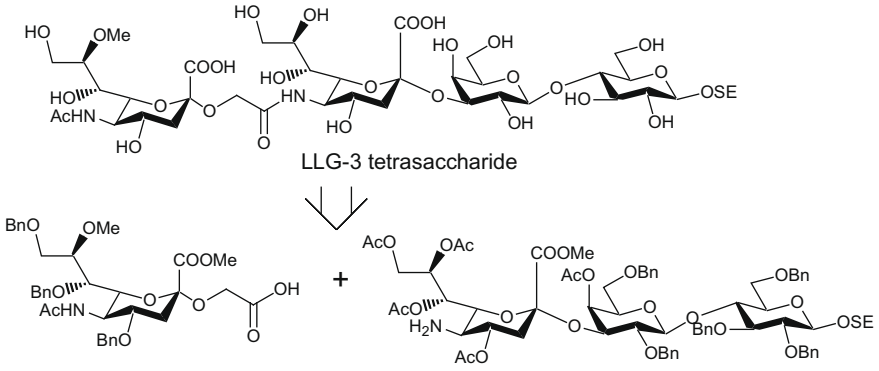
ABSTRACT

The first synthesis of the ganglioside LLG-3 tetrasaccharide, which has attractive biological activities as well as a unique structure, is described. A C8-methoxy decorated sialic acid building block was initially prepared and a glycolic acid moiety was then introduced by sialylation. Amide condensation between the sialyl glycolic acid and an amino group at C5 on the sialyllactoside unit afforded the fully protected LLG-3 tetrasaccharide. Finally, the desired tetrasaccharide part of LLG-3 was obtained after careful global deprotection.



1. Introduction

The structural diversity found in sialic acid and sialyl-glycolipids has great significance in biological systems and metabolic pathways of organisms.[1,2] Sialic acids identified in mammalian gangliosides mainly possess *N*-acetylneuraminic acid (Neu5Ac) and *N*-glycolylneuraminic acid (Neu5Gc) residues with α-(2→3/6) linkages to adjacent galactose, glucosamine, and galactosamine residues. Echinoderm gangliosides have more modifications of the sialic acid residues and exhibit various attachment linkages between the sialic acids and neighboring sugar residues. In some cases, distinctive sialic acids modified by acetylation and sulfation are known to be significant gateways for viral infections.[3] However, the actions of methylated sialic acids have remained unclear because of their presence in natural sources in small amount.

Another remarkable characteristic of this class of glycoconjugates is the connecting mode around the sialic acid residues.[4] One class of such gangliosides having di/tri-sialic acid residues with an α-(2→11) linkage has been found to possess nerve cell-stimulating and -protecting activities.[4,5] Due to the significant bioactivities of these unique sialic acid linkages, some pioneering studies toward the synthesis of these echinoderm gangliosides have been reported.[6]

The unique ganglioside LLG-3 (Scheme 1), isolated from the starfish *Linckia laevigata*,[5a] has potent nerve cell-stimulating activity for the neuron-like rat adrenal pheochromocytoma (PC-12) cell line through nerve cell growth factors (NGFs), which is of comparable magnitude to the mammalian ganglioside GM1.[5c,7] The distinctive tetrasaccharide sequence of LLG-3 contains a C8-methylated Neu5Ac (Neu8Me5Ac) terminus, which is connected to the *N*-glycolyl hydroxyl group (C11-OH) of the neighboring Neu5Gc residue. Because of its intriguing potent nerve cell-stimulating activities and its unique α-(2→11)-disialyl connection with

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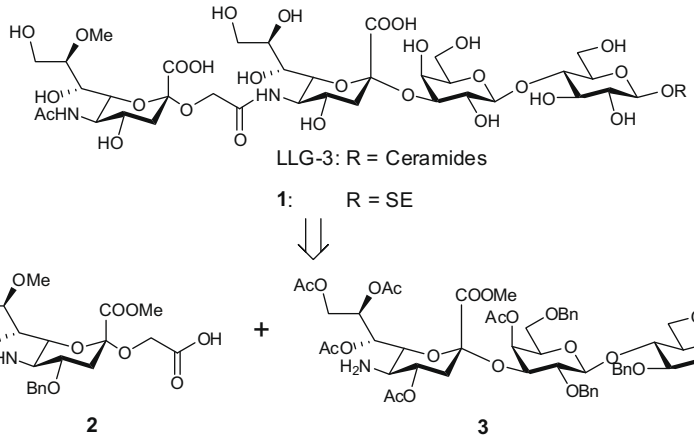
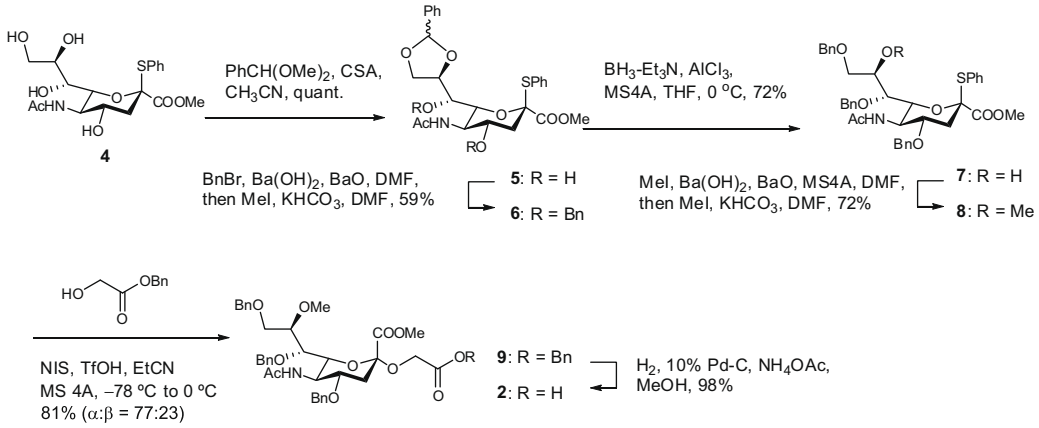


Scheme 1.



Scheme 2.

Neu8Me5Ac capping, we carried out the first synthesis of the LLG-3 tetrasaccharide, **1**.

2. Results and discussion

2.1. Synthetic plan

In our synthetic plan (Scheme 1), tetrasaccharide **1** is constructed by a [1+3] amide condensation between carboxylic acid **2** and the sialyllactoside **3**, which contains an amino group at C5. This approach avoids a poorly selective sialylation reaction later in the synthetic route.[8] To synthesize carboxylic acid unit **2**, the glycolic acid is liberated after introduction of the 8-methoxy sialic acid building block **8** by a glycosylation. The GM3-type trisaccharide **3** is synthesized by sialylation between the reactive *N*-Troc protected sialic acid building block **10** and lactose acceptor **11**,[9] followed by removal of the *N*-Troc group.

2.2. Synthesis of building block (2)

Synthesis of the Neu8Me5Ac carboxylic acid building block **2** began with β-phenyl thiosialoside **4**[10] (Scheme 2). The C8- and C9-hydroxyl groups were first protected as a benzylidene acetal by treatment with benzaldehyde dimethylacetal and camphorsulfonic acid to give **5** quantitatively. The C4/C7 di-*O*-benzyl intermediate was successfully produced by subsequent treatment with a combination of benzyl bromide, $Ba(OH)_2$ and BaO, conditions that were able to avoid undesirable N-benzylation.[11] Subsequent reprotection of the hydrolyzed carboxylic acid with a methyl group produced **6** in 59% yield. Reductive benzylidene ring opening with $BH_3{\cdot}Me_3N$ and $AlCl_3$ produced **7** in 72% yield.[12] To introduce a methyl group on the liberated C8-hydroxyl group, **7** was treated with MeI, $Ba(OH)_2$, and BaO in the presence of 4 Å molecular sieves; re-esterification produced the desired **8** in 72% yield. The obtained Neu8Me5Ac building block **8** was subjected to sialylation reactions with commercial benzyl glycolate in the presence of NIS and TfOH to afford **9** in 81% yield (α/β = 77/23, α-anomer: $^3J_{C1-H3ax}$ = 4.9 Hz).[13] The desired carboxylic acid **2** was produced by catalytic hydrogenation for **9α** with 10% Pd–C and excess NH_4OAc in 98% yield.[14]

2.3. Synthesis of LLG-3 tetrasaccharide (1)

The synthesis of the target LLG-3 tetrasaccharide **1**, involving formation of the full sequence through stepwise glycan chain elongations as well as subsequent global deprotections, is depicted in Scheme 3. To form the sialyllactoside **12** on a gram scale, initial sialylation between the 1-*O*-trimethylsilylethyl (SE) protected lactose acceptor **11**[15] and the potent *N*-Troc sialic acid building donor **10**,[16] which was activated by NIS–TfOH in CH_3CN, produced an inseparable crude mixture of trisaccharide. This mixture was acetylated[17] to give **12α** (α/β = 6:1, α-anomer: $^3J_{C1-H3ax}$ = 4.1 Hz)[18] in 67% yield. The desired trisaccharide was readily separated by silica gel chromatography after acetylation.

The *N*-Troc group of **12** was reductively removed using Zn powder in AcOH to afford the desired amine **3** in 85% yield. The liber-